

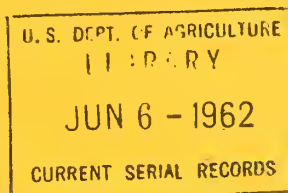
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EXTRACTING, PROCESSING, AND STORING SOUTHERN PINE POLLEN

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EXTRACTING, PROCESSING, AND STORING SOUTHERN PINE POLLEN

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Four years of experiments at the Southern Institute of Forest Genetics have confirmed and amplified prescriptions of previous writers for successful handling of pine pollen. The warm, humid climate of southern Mississippi, conducive to rapid deterioration of pollen, afforded rigorous conditions for the Institute's work. The purpose of this paper is to report results of the tests and put them into form for practical application.

Current prescriptions for extraction, processing, and storage are summarized immediately below. In effect, they comprise the Institute's extensions and modifications of earlier and less comprehensive suggestions by Wakeley and Campbell (1954), Mergen *et al.* (1955), and others. Later pages discuss the experimental basis for recommending these procedures in preference to others and suggest possible refinements that await further research.

RECOMMENDATIONS IN BRIEF

Optimum stage for collection.—Ideally, strobili should be plucked just before they are ready to shed pollen. For any given tree the best indication of such maturity is actual pollen release by some strobili. Ripeness is also indicated when a pasty yellow juice instead of a clear one can be squeezed from the strobili.

Contaminating pollen may be floated off by immersing strobili in an overflowing container of water, after which the wet strobili can be partially dried with paper toweling.

Forcing.—Less mature strobili can be nursed into shedding if pollen nuclei have reached a sufficiently advanced stage. Branches bearing strobili that are nearly ready to shed need only be stored in a warm place for a few days, with stems in water. When shedding begins, the strobili are plucked for extraction.

If pollen is desired before natural shedding, very immature strobili may be forced. To avoid premature drying, clusters should be covered with plastic or sausage-casing bags until they begin to shed. Some viable pollen will develop from material collected in the tetrad stage, but germinability increases with maturity of the strobili.

Another way of forcing pollen is to cover the male strobili on the tree at the same time that the female flowers are isolated for pollination, and with the same type of sausage-casing

bags. This method is also useful in catching pollen from early maturing strobili on distant trees.

Extraction.—Both speed of extraction and quality of the pollen depend on exposing strobili to dry, warm (80° to 90° F.), moving air. They can be exposed either in closed kraft bags around which air is circulated briskly, or in screened funnels (covered with 10-oz. canvas) through which forced air is piped. In neither case should the strobili be piled more than one or two layers deep in the container. In damp, cold weather the circulating air requires artificial heating and dehumidification.

Before reuse, funnels and similar nondisposable extracting equipment should be decontaminated with rubbing alcohol, or by heating to 80° C. for 12 hours.

Drying.—For successful storage, pollen must be dry. It is dry if it pours smoothly like water, and falls freely from the walls of glass containers—i.e., is not sticky. It must also be free of sawfly larvae; these may be sieved out through voile cloth or 60-mesh screen, either during or after extraction. If relative humidity during extraction has been below 20 percent, the larvae will be automatically killed and dried up. Some drying of pollen can be accomplished during refrigeration, but this, even in combination with desiccants, may be insufficient.

Desiccation for 15 minutes at 5 mm. of vacuum will quickly reduce moisture content to a safe level. Silica gel (part tell-tale grade) is a convenient desiccant.

Storage.—Pollen keeps best in containers no bigger than 2-oz. pill bottles and filled no more than half way. These should be plugged loosely with cotton to avoid contamination, but not capped or sealed, and should be stored at freezing or slightly above. Accidental exposure to a humid atmosphere may be avoided by storing

EXTRACTING POLLEN FROM NORMALLY MATURED STROBILI

“It seems clear that standardization of humidity and temperature at which pollen is extracted must precede any careful work on pollen storage and barriers to species crosses” (Duffield, 1953).

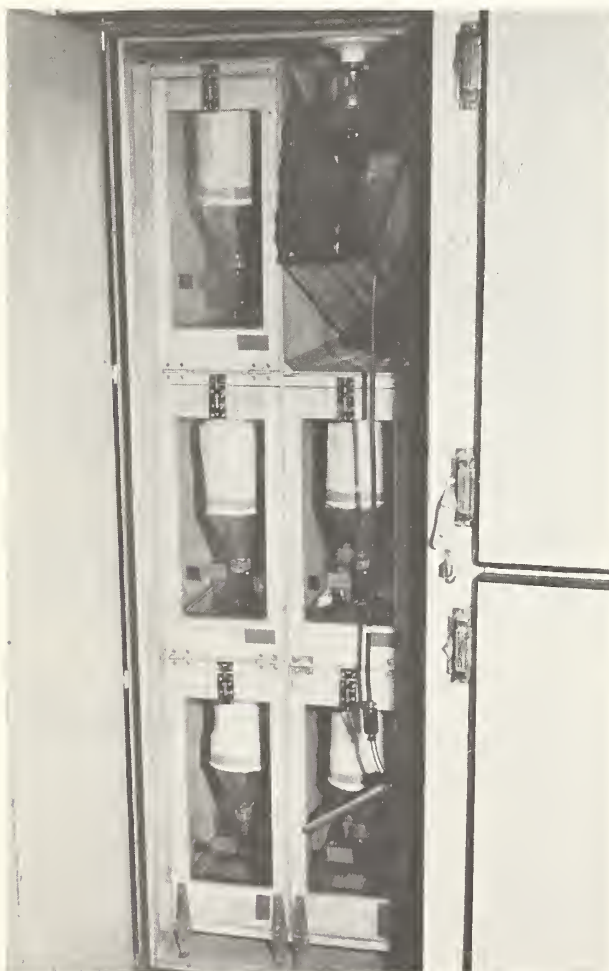


Figure 1.—Pollen extractor used for temperature-humidity tests.

over a saturated solution of potassium acetate to maintain humidity at 22 percent. (This solution requires 2 to 3 weeks to saturate before it can be used.)

Dispensing.—When part or all of the pollen in a bottle is withdrawn for use, water of condensation must not be allowed to vitiate past good storage practices. It must be allowed to evaporate before the pollen is manipulated.

Testing.—Before use, stored pollen should be tested for germination.

Agencies known to the writer use many types of extractors. Some enclose plucked strobili in sausage casings; others prefer wire cages in paper bags (Wakeley and Campbell, 1954). Some circulate dry air around extractors; others, including the Western Institute of Forest Genetics at Placerville, California, force dry filtered air through individual extractors by a blower and manifold system. The more primitive methods yield less pollen, and in damp weather increase the danger of spoilage.

When techniques for hastening the maturity of pine flowers become further advanced and specially designed batteries of environmentally controlled chambers become available, it will often be convenient to let strobili mature and shed while still attached to cut twigs (Barner and Christiansen, 1958; Worsley, 1959). The research to be described here, however, was concerned with the more general method of extracting pollen from plucked strobili that are placed in pollen-proof extractors through which filtered air is forced. This method produces dry, clean pollen, but to adapt it to the needs of the Southern Institute of Forest Genetics it was necessary to learn how various humidity-temperature combinations, and also the relative size of the strobili, affect extraction time, yield, moisture content, and insect pests of pollen from longleaf and slash pine (*Pinus palustris* Mill., *P. elliottii* var. *elliottii* Engelm.).

Methods.—Two series of tests were made, one in 1956 and one in 1957. Both measured the yield, moisture content, and germinability of pollen extracted at various combinations of temperature and relative humidity.

In 1956 two cabinets, each divided into 5 airtight compartments, were constructed in a refrigerator (fig. 1). One was heated to 20 and

the other to 25° C. Within the compartments, relative humidities of 25 to 65 percent were maintained with the aid of 1-liter dishes of sulfuric acid solutions of suitable concentrations (Wilson, 1921). Humidities were spot-checked with an electric psychrometer and found to be within 3 percent of expectation. The extractor in each compartment was covered with batiste, through which the appropriately conditioned air was pushed by a small electric fan. Each extractor was filled with 65 g. of strobili just beginning to shed pollen.

In 1957 both cabinets were kept at 25°, but a greater range of humidities was tested.

Pollen moisture was obtained from 1-gram samples oven-dried at 105° C. or above for 12 hours. Details of an improved method are given on page 6.

The medium used for pollen germination—0.75 percent agar and 10 percent sucrose—was that developed by Johnson (1943). From a 10-ml. pipette, 0.25 ml. of the unsterilized medium liquified by heat was dropped near each end of a microscope slide. When the agar medium had solidified, it was dusted with pol-

len from a loop of wire. The slide was then set vertically in a staining rack and placed in a moist chamber for incubation at 28° C. for 72 hours (fig. 2). A pollen grain was counted as germinated when the tube growth was as great as the grain's short dimension. Counts were made of 50 grains on each of two drops on separate slides. Results were comparable to those from liquid culture, but the method was much faster. Processing, from preparing the agar through recording the data, was performed at the rate of 45 counts per hour. The coefficient of variation was 7 percent.

Results.—After a 36-hour extraction period, dry-weight yields for slash pine pollen were 3.4 g. at 20° C. and 5.4 g. at 25°; longleaf yields were 4.2 and 5.0 g. (table 1). Relative humidity (RH) did not influence yields appreciably in either year. In most trials extractions below 45 percent gave best yields, but exact conditions cannot be specified. They apparently vary with the maturity of the strobili. Probably because of their larger mass, longleaf strobili required about 5 percent lower RH to make them comparable to slash pine strobili in yield and in pollen moisture content (MC).



Figure 2.—

A moist chamber for testing pollen germination. The racks hold 140 microscope slides, each with 2 drops of agar that have been lightly dusted with pollen.

Table 1.—*Dry-weight yields, moisture contents, and germination of pollen extracted from 65-g. samples of strobili in 36 hours at various combinations of temperature and humidity*¹

YIELDS

| Mean relative humidity (percent) | 1956 | | | | 1957 | |
|---|-------------------|--------|---------------|--------|--------------------|-----------------------|
| | Slash pine | | Longleaf pine | | Slash at 25° C. | Longleaf at 25° C. |
| | 20° C. | 25° C. | 20° C. | 25° C. | | |
| | ----- Grams ----- | | | | | |
| 10 | ... | ... | ... | ... | 3.7 | 3.2 |
| 15 | ... | ... | ... | ... | 2.8 | 3.1 |
| 20 | ... | ... | 4.1 | 5.0 | 2.7 | 2.6 |
| 25 | 3.0 | 5.2 | ... | ... | 2.7 | 2.7 |
| 30 | ... | ... | 4.2 | 5.2 | 2.8 | 2.9 |
| 35 | 3.1 | 5.2 | 4.3 | 5.0 | 3.4 | 2.8 |
| 40 | ... | ... | ... | ... | 2.9 | 2.5 |
| 45 | 3.7 | 5.7 | 4.2 | 5.0 | 2.5 | 2.8 |
| 50 | ... | ... | ... | ... | 2.2 | 2.3 |
| 55 | 4.0 | 5.5 | ... | ... | 2.2 | 2.6 |
| 60 | ... | ... | ... | ... | ... | ... |
| 65 | 3.3 | 5.4 | ... | ... | ... | ... |

MOISTURE CONTENTS

| | | | | | | |
|---------------------|-----|-----|-----|-----|-----|-----|
| ----- Percent ----- | | | | | | |
| 10 | ... | ... | ... | ... | 11 | 13 |
| 15 | ... | ... | ... | ... | 12 | 14 |
| 20 | ... | ... | 17 | 12 | 14 | 19 |
| 25 | 11 | 11 | ... | ... | 16 | 20 |
| 30 | ... | ... | 15 | 13 | 17 | 22 |
| 35 | 14 | 11 | 18 | 14 | 24 | 24 |
| 40 | ... | ... | ... | ... | 29 | 28 |
| 45 | 14 | 13 | 29 | 15 | 35 | 36 |
| 50 | ... | ... | ... | ... | 36 | 38 |
| 55 | 14 | 13 | ... | ... | 40 | 43 |
| 60 | ... | ... | ... | ... | ... | ... |
| 65 | 22 | 15 | ... | ... | ... | ... |

GERMINATIONS

| | | After 10 weeks | After 6 weeks |
|----|--|-------------------|------------------|
| 10 | [At 3 days after extraction, samples composited over all humidities germinated 96 to 98 per-cent.] | 97 | 95 |
| 15 | | 95 | 95 |
| 20 | | 97 | 95 |
| 25 | | 94 | 95 |
| 30 | | 97 | 93 |
| 35 | | 91 | 93 |
| 40 | | 69 | 94 |
| 45 | | 58 | 86 |
| 50 | | 59 | 85 |
| 55 | | 59 | 79 |

¹ Values in the table are means of pollen from 2 slash and 4 longleaf trees in 1956, and from 3 different slash and 4 different longleaf trees in 1957. Pollen from each tree was extracted separately.

In 1957 the decreased yields and increased MC may have been due to the fact that strobili of both species matured about a month earlier than usual.

After extraction, the 1957 pollen was put in sealed containers and kept at 5 to 10° C. until germination could be tested. The storage period for slash was 10 weeks; for longleaf, whose strobili generally mature about a month after slash, it was 6 weeks. Table 1 shows that serious deterioration may occur during even brief storage when extraction RH has been above 35 percent. The difference in storage periods may explain the greater loss in viability of slash pollen.

In 1957, a correlation was noted between the abundance of pollen sawfly larvae (*Xyela* sp.) and RH of the extractor. For example, at 20 percent RH (16 percent MC) there were only a few dried-up larvae, while at 55 percent RH the larvae were many and vigorous.

Extraction appeared to be more rapid in the forced-air extractors than in other types being tried at the same time. Because a gain of a few hours may permit crosses that otherwise would be impossible, this point was checked by filling kraft bags with the same quantities of strobili as forced-air extractors. Enough pollen for pollinations was produced in 12 hours by forced air, but not until after 24 hours in the closed kraft bags.

Wakeley and Campbell (personal correspondence) found that pollen extracted in sausage casing bags spoiled more easily than that extracted under similar conditions in kraft bags.

Everything considered, this research confirms that of Duffield (1953), who suggested extraction at 15 to 30 percent RH. Possibly 35 percent would be safe for southern pines under some conditions, but lower humidity affords insurance against damp weather or sawfly larvae.

ADJUSTING AND DETERMINING POLLEN MOISTURE

The work just discussed demonstrated that the quality of pollen is affected by moisture content. Information on pine pollen moisture content is meager. Ehrenberg (1961) reported

naturally shed *Pinus sylvestris* pollen to have 6 to 9 percent moisture. This is well below the 10 to 14 percent noted in this paper for "dry" extracted southern pine pollen. If the difference is a species characteristic, southern pine pollen may be the more difficult to store.

A simple and effective method is to dry pollen over silica gel for 15 minutes at 5 mm. of vacuum. This treatment has saved portions of wet southern pine pollen, other portions of which spoiled when placed over a desiccant in a refrigerator. In four successive annual tests, pollen vacuum-desiccated promptly after extraction has had consistently high germination after 1 year's storage.

Moisture contents best for storage can also be attained by regulating the humidity of the storage chamber, provided that the pollen is dry enough to keep until adjustment can occur. At the Southern Institute pollens placed in storage at MC's of greater or less than 12 percent have come to equilibrium at 12 percent in an atmosphere of 22 percent RH and 5 to 10° C.

Two new ways of determining pollen moisture contents have been devised. The first was suggested by the phenomenon that most pollen freshly extracted at 35 percent RH, and hence with MC of 22 percent or less, can easily be jarred loose from the walls of a glass container. While failure to stick to glass is a rough criterion, it may be reliable enough for practical purposes.

The second method is a revised oven-drying technique. Attempts to dry pollen by the standard method encountered difficulties similar to those reported by J. R. Hart¹ and co-workers (1959). Hart thereupon worked out a more accurate procedure with pollen sent him by the Southern Institute. It is a modification of techniques published by the U. S. Agricultural Marketing Service (1959).

For this technique, moisture dishes are necessary to protect the samples from losing or gaining moisture before and after the pollen is heated in the oven. Heavy aluminum dishes similar to those supplied by the Fisher Scientific Company as Special, Precision, No. 1631, are satisfactory. Before dishes are used, they should be dried for 1 hour at 100° C., cooled in a desiccator, and weighed. The desiccator

¹ Chemist, Field Crops and Animal Products Branch, Market Quality Research Division, Agricultural Marketing Service, U. S. Department of Agriculture.

should be airtight and should contain activated alumina or "Molecular Sieves" (Linde Air Products Company).

The procedure is to place portions (duplicates at least) of approximately 1 to 2 g. of the well-mixed sample in the tared moisture dishes, cover the dishes immediately, and weigh. When weights of the samples have been recorded, the dishes are set, with covers beneath, in a convection oven regulated to $100^{\circ} \pm 1^{\circ}$ C. All dishes should be on the same shelf, with the bulb of the oven thermometer as close as possible to them.

After 1 hour at 100° , the dishes are covered and transferred from oven to desiccator for cooling. They are weighed at room temperature. Replicate determinations of moisture should check within 0.2 percent.

With 6 samples varying from 10 to 14 percent in MC, Hart noted a maximum difference of 0.2 percent in MC between the Karl Fischer chemical method and the revised oven method. He found 130° C. unsuitable because pollen changed to a dark brown, which indicated loss of carbon dioxide in addition to water vapor (Hodge, 1953). Temperatures lower than 100° C. were not tested because of the likelihood of failure in driving off absorbed moisture. No change in weight occurred if drying was extended from 1 hour to 6 hours.

HASTENING POLLEN SHEDDING

A problem frequently confronting forest geneticists is how to obtain pollen from late-flowering trees for application to early-flowering trees of the same or other species. Often days or weeks separate the natural shedding of pollen on different trees.

Forcing may be regarded as a part of the extraction process; sometimes the extractor is also the forcing container. F. C. Cech, P. C. Wakeley, T. E. Campbell (personal communications), and the writer have forced pollen shedding as much as two weeks prematurely by covering the strobili on the tree with sausage-casing bags. This technique is also useful for catching uncontaminated pollen from early maturing strobili on trees that cannot be inspected frequently.

Mergen (1954) forced longleaf pollen shedding in the greenhouse by December grafting of scions bearing male strobili.

Cuttings with strobili may also be cultured with their bases in water or in nutrient solutions, and their tops exposed to one or another special environment. Thus, Santamour and Nienstaedt (1956) forced hemlock pollen shedding by liquid culture of twigs under 20 hours of light. Barner and Christiansen (1958) demonstrated the importance of maintaining a high humidity, which they attained by putting plastic bags over clusters of strobili on twigs in liquid culture. Worsley (1959) successfully forced shedding of pine pollen by exposing the strobili to either 600 watts at 1 m. for 18 to 22 hours with temperatures up to 27° C. or to 200 watts at 1.5 m. for 24 hours with temperatures up to 20° C. He used plastic bags to keep relative humidity at 80 to 100 percent for ripening and 60 to 70 percent for shedding.

The results reported here were obtained with cuttings in liquid cultures. Repetitions of elementary treatments previously described (Snyder, 1958), and included in the following discussion, have continued to save several days of ripening time, but no new treatments have been consistently worth while.

Methods and results.—Eleven trials were conducted in the greenhouse with shoots bearing male strobili: Slash and longleaf pine were studied in 1956; longleaf and loblolly in 1957; slash, longleaf, loblolly, and shortleaf in 1958; and slash, longleaf, and loblolly in 1959. "Plots" consisted of 1 shoot apiece from each of 6 to 8 trees, and treatments were applied in factorial design, with 2 replications. Maturities when treatment began varied with tree source, and ranged from early meiosis to immature pollen. Strobili that shed pollen in the greenhouse did so on an average of 3 weeks after collection of cuttings. Treatment effects were measured by strobilus growth. Maximum strobilus elongation resulted in shedding; less elongation was also considered as a valid measure of treatment effects, even when no pollen was released.

In 1956, statistically significant increases were found for 25° C. air temperature over 20° C.; continuous 40-watt fluorescent light 18 inches above the plants (probably as a heat effect) over no supplemental light; sugars plus biotocidal control (either 5 percent sucrose plus 0.75 percent ferbam or Floralife cut-flower preservative) over tap water control;

and boiling the base of the cuttings for 1 minute over no boiling.

Some additional results were that the boiling produced slash pine strobili of 20.7 mm. mean lengths as compared to 12.8 mm. for the un-boiled control, and that with longleaf the addition of sugars plus bioticial resulted in mean lengths of 46.1 mm. as compared to 37.8 mm. for the distilled water control.

The following additions to the distilled water cultures were without significant effect: air pumped in daily; bioticial mixture (streptomycin sulfate 1 percent, KMnO_4 0.05 percent, sodium pentachlorophenol 20 p.p.m., ferbam 0.75 percent); adsorbing charcoal (5 g./l.); soluble fertilizer (Hyponex); and 2,4,5-T (100 p.p.m. in 1 percent carbowax). Other ineffective treatments were sterilizing stems with saturated bromine water for 30 minutes, interrupting night by 1 hour of supplementary light, cutting the stems under water, and immersing the stems in 50°C. water for 5 minutes.

Trials in years subsequent to 1956 failed to duplicate the large differences originally observed with bioticials and boiling. In all 11 trials, however, a cut-flower preservative did consistently induce strobilus growth over that obtained with distilled water. Boiling gave variable results and reduced strobilus growth on loblolly significantly below that of the control.

Miscellaneous observations were (1) that in all three years of testing, the tap water, which had a high iron content, produced loblolly strobilus growth superior to that achieved with distilled water; (2) that removal of needles 10 days prior to pollen shedding or at the beginning of shedding was not deleterious but that removal when the cuttings were taken was harmful; and (3) that viable pollen could seldom be recovered when forcing was begun earlier than the tetrad stage of meiotic division. When forcing was started at the tetrad stage the pollen germination was 50 percent; at the first prothallial stage, 88 percent; at complete maturity and onset of normal discharge, 94 percent.

The forcing problem may be a double one, entailing recovery of pollen separately from improvement of its viability (Christiansen,

1960). Cumming and Righter (1948) say that the chances of getting good pine pollen are improved by deferring the plucking of strobili until the first prothallial nucleus of the pollen grain is microscopically visible.

STORING POLLEN

Southern pine pollen is at present being stored in quantities ranging from a gram to a gallon, in sealed or unsealed containers, and wet or dry—with variable results as a consequence. Both in research and in large-scale tree improvement there is obvious need for reliable methods of maintaining pollen in highly viable condition for one or more years.

In addition to storage at temperatures which can be obtained in a household refrigerator, there are at least two other methods for enhancing preservation. One of these, surface sterilization, has been practiced by Helmers (1950), Sato and Muto (1955), and Tulecke (1954, 1960). Tulecke has stored ginkgo pollen successfully for 4 years. Another way is to deep-freeze or vacuum-store, as exemplified by the work of Barber and Stewart (1957), Duffield and Callahan (1959), and Ehrenberg (1961). The last two papers give evidence that pollen from deep-freeze storage sets more seed than equally viable pollen kept at ordinary refrigerator temperatures. The Southern Institute of Forest Genetics has adapted the freeze-drying methodology of King (1959, 1961), whose research was stimulated in part by Hesselstine and Snyder (1958). The equipment is expensive, however, and the expected benefits are not yet fully confirmed.

For storage of pollens of most western pines at ordinary refrigerator temperatures, investigators, including Duffield and Snow (1941), Johnson (1943), and Duffield (1954), have found optimum humidities varying from 10 to 50 percent depending on the species. In recent research with western pine, Fechner *et al.* (1960) reported 35 percent germination at 50 percent RH after 6 years, while Stanley *et al.* (1960) obtained approximately similar germination after 15 years at 10 percent RH. Worsley (1959) found no loss in germination of *Pinus sylvestris* during 3 years at 10 percent RH and 0° C. For prolonged storage, Stanley *et al.* stress the advantage of 0° over 5° C.

Research at the Southern Institute of Forest Genetics has been limited to pollens with different moisture contents stored at 5 to 10° C.

Sealed vs. unsealed containers.—It was hypothesized that an optimum storage RH might be indirectly detected by finding the pollen MC resulting in best germination when storage is in sealed tubes, and equating this value with the RH equivalent. Unsealed containers were considered controls.

Pollens with known initial MC's were derived by bulking samples from the 1956 extraction trial (table 1). For slash pine, four 1-g. aliquots from each MC were placed in separate 15-ml. shell vials. Two vials were scheduled to be tested for germination after 22 months and two after 32 months. Before storage, the mouth of one member of each pair was corked and coated with hot paraffin; the other member was left unsealed and exposed during stor-

age to the atmosphere above a saturated solution of potassium acetate. This solution is listed (by Winston and Bates, 1960) as giving an RH of approximately 22 percent; it requires several weeks to saturate before it can be used. The pollens and the bottles were weighed both before and after storage to determine MC after storage.

Longleaf pollens were treated similarly except that storage in sealed tubes was limited to 22 months and MC was not read.

Percentage of germination for each treatment was determined by counting 300 grains (50 grains on each of 6 slides).

Germination and final MC are shown in table 2. In the sealed tubes most MC's remained constant or declined only slightly. The one exception was in the 22-percent initial MC, where the pollen fermented, broke the seal, and fell to a much lower final MC.

Table 2.—*Final moisture contents and germination of pine pollens collected in 1956 and stored at 5 to 10° C.*

| STORAGE IN UNSEALED TUBES AT 22 PERCENT RELATIVE HUMIDITY | | | | | | | |
|---|-----------------|-----------|--------------------|-----------|-----------------------------|--------------------|-----------|
| Initial moisture content (percent) | Slash pine | | | | Longleaf pine | | |
| | Moisture at— | | Germination at— | | Moisture at 32 months | Germination at— | |
| | 22 months | 32 months | 22 months | 32 months | | 22 months | 32 months |
| ----- <i>Percent</i> ----- | | | | | | | |
| 9 | ... | ... | ... | ... | 11 | 84 | 78 |
| 10 | ... | ... | ... | ... | 12 | 87 | 79 |
| 11 | 11 | 11 | 89 | 83 | 12 | 85 | 72 |
| 12 | ... | ... | ... | ... | 12 | 82 | 79 |
| 13 | 12 | 12 | 88 | 83 | ... | ... | ... |
| 14 | 11 | 11 | 84 | 86 | 13 | 82 | 84 |
| 15 | 12 | 12 | 88 | 88 | 13 | 80 | 83 |
| 22 | 15 | 12 | 88 | 91 | ... | ... | ... |
| 29 | ... | ... | ... | ... | 13 | 65 | 67 |
| STORAGE IN SEALED TUBES | | | | | | | |
| 9 | ... | ... | ... | ... | ... | 88 | ... |
| 10 | ... | ... | ... | ... | ... | 90 | ... |
| 11 | 11 | 11 | 91 | 79 | ... | 92 | ... |
| 12 | ... | ... | ... | ... | ... | 85 | ... |
| 13 | 13 | 12 | 86 | 80 | ... | ... | ... |
| 14 | 13 | 12 | 82 | 72 | ... | 79 | ... |
| 15 | 14 | 14 | 79 | 64 | ... | 73 | ... |
| 22 | 11 | 8 | 0 | 0 | ... | ... | ... |
| 29 | ... | ... | ... | ... | ... | 0 | ... |

When stored at 22 percent RH, most of the pollen came to an equilibrium MC of about 12 percent by 22 months; some vials lost and some gained, according to whether they were originally greater or less than this value. Germination as high as 91 percent was obtained after 32 months.

Optimum MC for storage appeared to be determined by and to vary with storage conditions, as follows:

For 32 months of unsealed storage in 22 percent RH, initial MC's of 9 to 22 percent insured germination of at least 72 percent. MC for best germination appeared to vary with length of storage, being 9 to 13 percent for 22 months and 14 to 22 percent for 32 months.

For sealed storage the range from 9 to 13 percent produced germinations about equal to those in unsealed storage, but at higher MC deterioration was greater. The difference in response from unsealed pollen is reflected in a highly significant *storage method* \times *MC* interaction term in the analysis of variance. Visser (1955) stated from his work with several fruit, vegetable, and flower species that "Storage at 2-4° C. in sealed tubes appears to have no or little advantage over storage with an optimal relative humidity."

In 1957, pollens at various MC's were stored both sealed and exposed to 22 percent RH; temperatures were 5 to 10° C. The initial yield, MC, and germination means from the extraction data appear in table 1, and germination percentages after 22 months are in table 3. The results confirm those of 1956 in that sealing was detrimental with high MC's. In this test, though, even unsealed pollen suffered some reduction in germination if placed in storage at an initial MC above 25 percent.

Seed-setting ability was tested by Campbell and Wakeley (1961) in west-central Louisiana. Results (table 4) indicated that pollen of the sealed series lacked the fertilizing ability of the unsealed series, even when germination percentages were equal. Roughly 15 percent as many full seeds per collected cone were obtained from the best of the 2-year-old pollen as from fresh pollen. This is comparable to the 13 percent obtained with 1-year-old pollen in interspecific pine crosses (Wright and Gabriel, 1958), but contrasts with the 68 percent obtained with intraspecific pine crosses (Wright, 1959). Some deterioration may have occurred when the pollen was shipped from Mississippi to Louisiana.

Amount per bottle.—That pollen germination is sometimes poor if large volumes are stored in a bottle was shown by Snyder (1958). Worsley (1959) noted reduced germination in samples drawn from the lower portion of a test tube filled more than 5 cm. deep. The re-

Table 3.—*Germination of pine pollens collected in 1957 and stored at 5 to 10° C. for 22 months*

| Initial moisture content (percent) | Slash pine | | | Longleaf pine | | |
|------------------------------------|------------|---------------|-----------------------|---------------|---------------|-----------------------|
| | Samples | Sealed | Unsealed ¹ | Samples | Sealed | Unsealed ¹ |
| | Number | -- Percent -- | | Number | -- Percent -- | |
| 9-10 | 4 | 47 | 62 | 3 | 72 | 67 |
| 11-15 | 9 | 33 | 64 | 6 | 29 | 59 |
| 16-20 | 5 | 0 | 52 | 7 | 0 | 61 |
| 21-25 | 2 | 0 | 52 | 8 | 0 | 64 |
| 26-30 | 2 | 0 | 65 | 4 | 0 | 56 |
| 31-35 | 3 | 0 | 42 | 1 | 0 | 61 |
| 36-40 | 0 | ... | ... | 5 | 0 | 25 |
| 41-45 | 2 | 0 | 0 | 3 | 0 | 29 |
| 46-50 | 1 | 0 | 0 | 1 | 0 | 16 |
| 51-55 | 2 | 0 | 0 | 0 | ... | ... |

¹ In atmosphere at 22 percent RH.

Table 4.—*Seeds recovered from controlled pollinations of longleaf pine with pollen of varying germinations*¹

| Germination of pollen when applied (percent) | Full seeds | | Proportion full |
|--|------------------|--------------------------|-----------------|
| | Per matured cone | Per strobilus pollinated | |
| | - Number - | | Percent |
| Stored unsealed | | | |
| 65 | 11 | 3 | 88 |
| 74 | 14 | 3 | 22 |
| Stored sealed | | | |
| 58 | 0 | 0 | 0 |
| 76 | 6 | 1 | 11 |
| 87 | 5 | 2 | 12 |
| 92 | 13 | 2 | 22 |
| Fresh pollen (control) | | | |
| 92 | 91 | 26 | 96 |

¹ The stored pollen was from a single Mississippi lot extracted and stored for 2 years in different ways; the fresh control was from two Louisiana trees.

sults reported here extend observations of Snyder (1958) to 22 months.

Samples having 12, 29, and 51 percent MC were stored for 10 and 22 months in 24-ml. vials filled to varying heights (table 5). Storage was at 5 to 10° C. and 22 percent RH. Germination percentages were calculated by counting 300 grains (3 bottles per treatment times 2 slides per bottle times 50 grains per slide).

With wet pollen (51 percent MC), storage in small amounts offered some protection for 10 months. Germination was 73 percent for pollen stored in amounts of 4 ml. per 24-ml.

vial, but dropped to 47 percent when amount per vial was increased to 20 ml. (table 5). The explanation probably is that the smaller amounts dried more efficiently during storage. The differences for 10 months of storage are highly significant, but after 22 months all pollen stored at 51 percent MC was dead.

Amount per bottle did not significantly affect the keeping quality of pollen at 12 or 29 percent MC.

DISPENSING STORED POLLEN

Mergen *et al.* (1955) warned against the wetting of stored pollen by water condensing on the cold glass walls of a container removed from refrigeration. Common observations that pollen apparently deteriorates when refrigeration is interrupted to remove samples prompted a study of the problem at the Southern Institute. The results showed that a few minutes of mishandling during withdrawal of pollen for current use may vitiate all the good effects of years of otherwise perfect storage.

Five-gram samples were stored at 5 to 10° C. in 2-oz. pill bottles. No pollen was actually taken out, but withdrawals were simulated by shaking the bottles. Pollen was "dispensed" in this way on two successive days; rainy periods in spring were chosen in order to make the tests as severe as possible. Pollen of two species was represented—slash at 50 percent initial MC, and longleaf at 12 percent. Slash was tested for germination 10 months after it had been dispensed; longleaf was tested after storage for 22 months, during which time it had been dispensed in two successive springs. Germination was calculated from counts of

Table 5.—*Germination, after 10 and 22 months of storage, by quantity of pollen per container and moisture content.*

| Ml. of pollen per 24-ml. vial | 51 percent moisture | | 29 percent moisture | | 12 percent moisture | |
|-------------------------------|---------------------|-----------|---------------------|-----------|---------------------|-----------|
| | 10 months | 22 months | 10 months | 22 months | 10 months | 22 months |
| | ----- Percent ----- | | | | | |
| 4 | 73 | 0 | 78 | 69 | 94 | 90 |
| 8 | 58 | 0 | 76 | 67 | 95 | 87 |
| 12 | 54 | 0 | 77 | 69 | 90 | 87 |
| 16 | 49 | 0 | 80 | 66 | 89 | 88 |
| 20 | 47 | 0 | 79 | 70 | 91 | 87 |

300 grains per treatment (50 grains on each of 6 slides).

Table 6 summarizes and compares the various treatments. Treatment 1 consisted of removing an unsealed bottle from the refrigerator, allowing it to reach room temperature, "dispensing" some pollen, and restoring it to refrigeration still unsealed. Treatment 2 sealed a similar, previously unsealed bottle before it was brought out of the refrigerator but unsealed it again upon return. Treatments 3 and 4 resembled 1 and 2, except that storage was in sealed bottles both before and after dispensing. Bottles for treatment 5, a sealed control, and treatment 6, an unsealed control refrigerated at 22 percent RH, were never dispensed,

but were brought to room temperature and then refrigerated. Treatment 7 was an unsealed control that was never removed from the refrigerator.

For slash pollen there were duplicate bottles for each treatment. One of these was put back into the refrigerator at night; the other left out until the end of the second day of dispensing. Longleaf pollen, in triplicate bottles, had a third treatment during the 2-day dispensing periods, viz., putting the bottle back into the refrigerator immediately after the dispensing.

Results indicate the benefit of minimizing exposure to air temperature during the 2-day dispensing period. Losses were heavy, however,

Table 6.—*Germination of pine pollen (slash initially at 50 and longleaf at 12 percent moisture) after dispensing and subsequent storage*¹

| Storage during and after dispensing | Treatment after removal from storage until dispensing ² | Treatment between first- and second-day dispensing | | | | |
|---------------------------------------|--|--|----------------------------------|---|-----------------------|----------------------------------|
| | | Slash pollen | | Longleaf pollen | | |
| | | Refrigerated at night | At room temperature continuously | Refrigerated immediately after dispensing | Refrigerated at night | At room temperature continuously |
| ----- Percent ----- | | | | | | |
| 1. Unsealed | Unsealed | 74 | 72 | 80 | 78 | 74 |
| 2. Unsealed | Sealed after removal from refrigerator until dispensed | 72 | 70 | 82 | 77 | 77 |
| 3. Sealed | Unsealed after removal from refrigerator until dispensed | 36 | 41 | 58 | 1 | 9 |
| 4. Sealed | Sealed until dispensed | 44 | 43 | 86 | 11 | 7 |
| 5. Sealed (control) | Sealed—never dispensed | 30 | 19 | 87 | 89 | 83 |
| 6. Exposed to 22 percent RH (control) | Exposed to 22 percent RH—never dispensed | 73 | 74 | 82 | 74 | 76 |
| Mean | | 55 | 53 | 79 | 55 | 54 |
| 7. Unsealed (control) | Never removed from refrigerator | 75 | | 80 | | |

¹ Total storage of 10 months for slash pollen, 22 months for longleaf.

² No pollens were dispensed until they had reached room temperature.

only for longleaf pollen that was sealed in storage but left out of the refrigerator for 2 days in each of the 2 seasons. Germination for these lots was from 1 to 11 percent, as compared to 58 and 86 percent for pollen returned to the refrigerator immediately after being dispensed.

Eliminating condensation water by sealing bottles when they were taken from the refrigerator was effective only with the sealed-storage lots (cf. treatments 3 and 4, table 6). The lack of benefit with unsealed storage is probably explained by the subsequent removal of the condensed water through the desiccating action of the refrigerator.

TECHNICAL SUMMARY

Results from 4 years of experiments show that pollen can be extracted several days earlier than normal if strobilus-bearing cuttings are cultured in liquid. Best germination has been obtained when cuttings were taken after meiotic division had reached the tetrad stage. Low relative humidities in the extraction chamber have speeded recovery from fully matured strobili.

Either the use of dry air during extraction or vacuum desiccation after extraction reduced pollen moisture to levels safe for storage.

Relative humidity and temperature of air forced over strobili during extraction determined several characteristics of recovered pollen. Yield was satisfactory between 10 and 45 percent RH and was better at 25° than at 20° C. Pollen sawfly larvae were dried up

at 20 percent RH (16 percent MC) and below. Pollen MC varied from 11 to 43 percent between extraction humidities of 10 and 65 percent.

Moisture content low enough for safe storage (about 22 percent) is indicated when pollen does not stick to the walls of a glass container. A technique for accurate moisture determination by the oven-dry method is reported.

With pollen of 51 percent MC, storage of small amounts in large (24 ml.) unsealed vials offered partial protection for 10 months, because it afforded opportunity for drying. At more nearly optimum storage MC's of 29 and 12 percent, degree of filling did not significantly affect keeping quality.

When lots with initial MC's of 9 to 22 percent were stored at 22 percent RH or exposed to the atmosphere of a household refrigerator, germination up to 91 percent was obtained after 32 months. Deterioration resulted at 29 percent MC and above.

Sealing the containers caused spoilage when MC was 22 percent or higher. Even when germinability was equal, seed set was greater from pollen stored in open than in sealed containers.

Sealing for restorage was detrimental if moisture was allowed to condense on the cold walls of the container while pollen was being dispensed.

From these and other studies some general suggestions for pollen handling have been formulated (see page 1).

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